ANNUAL REPORT OF BIOLOGICAL ACTIVITIES

~ 2001 ~

U.S. Fish and Wildlife Service

Northeast Fishery Center
Lamar, Pennsylvania

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In fiscal year 2001, the Northeast Fishery Center (NEFC) progressed toward the goals of establishing a regional genetics laboratory on-site and performing advanced population ecology studies. Applied research continued in many areas of fish culture technology with inter-jurisdictional species such as American shad, Atlantic salmon, and Atlantic sturgeon. We completed construction of an experimental ozone water treatment plant which will enable fish culture and experimentation with pathogen-free water. Important advancements were made in tank-spawning of American shad by building upon previous years= experimentation. The first field test of a new fish marking technology developed at NEFC was performed in Maine with endangered Atlantic salmon stocks. Experience was gained in diagnosis and management of Infectious Salmon Anemia virus (ISAv), a pathogen which was recently isolated in samples taken from Atlantic salmon in maritime culture in waters of Maine and which represents a potential threat to Atlantic salmon restoration efforts in Region 5. Staff members also put much time and effort into constructing a flow-through holding system for mature striped bass on the banks of the Hudson River and performing the second study of mortality associated with recreational angling of striped bass in a cooperative project with the state of New York.

STUDIES PERFORMED

Study Number and Title:

(Previously unreported results from 2000 experiments):

LM-00-06 Comparative efficacy and an evaluation of the stress response of Atlantic sturgeon *Acipenser oxyrinchus* to three fish anesthetics.

(Current fiscal year, 2001 studies):

LM-01-01 Determination of gonadal maturity in 5 yr-classes of domestic Atlantic sturgeon.

LM-01-02 Analysis of lipofuscin pigment concentration in brain tissue from progressive size classes of horshoe crabs *Limulus polyphemus* as a possible indicator of age.

~~LM-01-03 The use of ultrasonic tracking to locate Atlantic sturgeon spawning areas of the Delaware River and Bay~~. UNFUNDED

LM-01-04 Growth comparison of Atlantic salmon parr to determine effectiveness of three commercial diets.

LM-01-05 Comparison of mortality between calcein-marked and unmarked Atlantic salmon fry stocked in the Sheepscot River, Maine.

LM-01-06 Effect of Hormone Selection and Water Salinity upon Tank Spawning Performance of American Shad.

LM-01-07 Mortality associated with catch and release angling of striped bass in the Hudson River.

LM-01-08 Preliminary evaluation of calcein absorption via the gastrointestinal tract of juvenile Atlantic salmon

OTHER BIOLOGICAL AND RELATED INVESTIGATIONS PERFORMED:

LM00A Fish Health Inspection/Monitoring/Diagnostic Services

LM01B Licensing agreement for calcein detection devices

LM01C Ozone water treatment plant construction

LM01D Fish Health Inspection/Monitoring/Diagnostic Services

LM01E Participation in the National Wild Fish Health Survey

LM01F Incidence and Prevalence of Infectious Salmon Anemia in Sea Run Penobscot River Atlantic Salmon held at Craig Brook NFH for Broodstock

LM01G Participation in Maine Fish Health Advisory Board concerning ISAv Issues

LM01H U.S. Fish and Wildlife Service Fish Health Procedures Handbook

LM01I Quality Assurance/Quality Control for ISAv Samples and Diagnostic Techniques

LM01J Fish Health Extension Services

STUDIES IN WHICH THE CENTER COOPERATED:

Effect of temperature, oxygen, dietary phosphorus, and vitamin D3 on phosphorus levels in effluent from experimental culture of rainbow trout. - *Reli Coloso, Department of Pharmacology and Physiology, UMDNJ - New Jersey Medical School, Newark, NJ*

Tagging horseshoe crabs to determine spawning frequency and beach fidelity in Delaware Bay. - *Maryland Fisheries Resource Office, USFWS, Annapolis, MD.*

The ecology of whirling disease *(Myxobolus cerebralis*) in Pennsylvania.- *Adam Kaeser, Pennsylvania State University*

PUBLICATIONS:

Coloso, R.M., S.P. Basantes, K.King, M.A. Hendrix, J.W. Fletcher, P.Weis., and R.P. Ferraris. 2001. Effect of dietary phosphorus and vitamin D3 on phosphorus levels in effluent from experimental culture of rainbow trout. Aquaculture 202:145-161.

Jodun, W.A., M.J. Millard, and J.W. Mohler. 2002. The effect of rearing density on growth, survival, and feed conversion of juvenile Atlantic sturgeon. North American Journal of Aquaculture. 64:10-15.

King, K., and P. Farrell. 2002. Sensitivity of juvenile Atlantic sturgeon to three theraputic chemicals used in aquaculture. The North American Journal of Aquaculture 62:60-65

Mohler, J.W., M.J. Millard, and J.W. Fletcher. 2002. Predation by captive wild brook trout on calcein-marked versus nonmarked Atlantic salmon fry. North American Journal of Fisheries Management. 22:223-228.

TECHNICAL INFORMATION LEAFLETS:

LM-00-08. Selected review of literature pertinent to impacts of incubation temperature on Atlantic salmon fry.

TECHNICAL REPORTS:

Fletcher, J. 2001. Relative efficacy of 2 hormones for inducing spawning in American shad. In: The Annual Progress Report for Restoration of American shad to the Susquehanna River.

Millard, M.J., J. Mohler, A. Kahnle, A. Cosman, K. Hattala, and W. Keller. 2001. Mortality associated with catch and release angling of striped bass in the Hudson River. Reportr to: NY State Dept. of Environmental Conservation, Hudson River Fisheries Unit, New Paltz, NY.

Millard, M., S. McCormick, J. Mohler, J. Fletcher, M. O=Dea, D. Lerner, and A. Moeckel. 2001. Evaluating sedation as a means to suppress the stress response associated with handling and transport of American shad. Chapter 5: Annual Report to the Susquehanna River Anadromous Fish Restoration Committee, Susquehanna River Commission, Harrisburg, PA.

FORMAL PRESENTATIONS:

Coll, John. - The National Wild Fish Health Survey-Implementation and Findings, and New Technologies in Fish Health. Vermont Department of Fish and Wildlife Fish Culture Workshop. January 29-30, Grand Isle, Vermont.

Coll, John. - Atlantic Salmon Swim Bladder Sarcoma Virus, Management of a Newly Reported Virus in Imperiled Feral Stocks. 26th Annual Eastern Fish Health Workshop. April 23-26, 2000, Shepherdstown, West Virginia.

Fletcher, John - Relative efficacy of 2 hormones for inducing spawning in American shad. Susquehanna River Anadramous Fish Restoration Cooperative Technical Committee. Jan. 17. Harrisburg, PA.

Fletcher, John - Evaluating sedation as a means to suppress the stress response associated with handling and transport of American shad. American shad Marking Coordination and Culture Meeting. Jan 24. Annapolis, MD.

Hendrix, Michael - The NE Fishery Center at a glance. Presentation to Region 5 Directorate. May 31. Hadley, MA.

Jodun, Wade - Growth and feed conversion of sub-yearling Atlantic sturgeon at three feed rates. Apr. 25. Fifty-seventh annual NE Fish and Wildlife Conference. Saratoga Springs, NY.

Jodun, Wade -The effect of rearing density on growth, survival, and feed conversion of juvenile Atlantic sturgeon. Apr. 25. Fifty-seventh annual NE Fish and Wildlife Conference. Saratoga Springs, NY.

Jodun, Wade - Effect of iodophor concentration and duration of exposure during water hardening on survival of Atlantic salmon eggs. Jan. 25. Connecticut River migratory fish restoration research forum. Hadley, MA.

Millard, Michael - Catch and release mortality rates in striped bass fishery of the Hudson River. Apr. 23. Fifty-seventh annual NE Fish and Wildlife Conference. Saratoga Springs, NY.

Millard, Michael. Mortality associated with catch and release angling of striped bass in the Hudson River. August, 2001. Annual Meeting of American Fisheries Society, Phoenix, AZ.

Millard, Michael. Using sedation to suppress the stress response associated with handling and transport of American shad. August, 2001. Annual Meeting of American Fisheries Society, Phoenix, AZ.

Millard, Michael. The biology and politics of horseshoe crab management on the Atlantic coast. May, 2001. Pennsylvania State University, School of Forest Resources, Dept. of Wildlife and Fisheries Science seminar.

Mohler, Jerre - Status of calcein marking technology. Jan. 25. Connecticut River migratory fish restoration research forum. Hadley, MA.

Mohler, Jerre - Effects of short term light deprivation upon milt production of feral Atlantic. Jan. 25. Connecticut River migratory fish restoration research forum. Hadley, MA.

Mohler, Jerre - Predation by captive wild brook trout on calcein-marked vs non-marked Atlantic salmon fry. Apr. 25. Fifty-seventh annual NE Fish and Wildlife Conference. Saratoga Springs, NY.

Mohler, Jerre - Progress report on calcein marking. Feb 28. Maine Atlantic salmon technical advisory committee meeting. Waterville, ME

Mohler, Jerre - Marking fish with calcein - a new technology developed at the NE Fishery Center-Lamar, PA. Presentation to Region 5 Directorate. May 31. Hadley, MA.

Mohler, Jerre - The NE Fishery Center and Lamar Fish Health Unit - what we can do for you. Region 5 Biologists workshop. Oct. 17. National Conservation Training Center. Shepherdstown, WV.

National Committee participation:
Coll, John and Patricia Barbash.- Served on the National Fish Health Policy Revision Committee to re-write the National Fish Health Policy and Procedures handbook.

Coll, John.- Served on the National Title 50 Revision Committee to re-write Title 50 regulations for importing fish into the U.S.

Fletcher, John.- Served on the national ad-hoc committee to review and report to the Director upon the potential of MAXIMO^{7} software for tracking maintenance and property at USFWS facilities.

Fletcher, John.- Served as NEFC representative in Service and Maintenance Management System (SAMMS). NEFC was one of 3 fishery units nationally selected to pilot test the MAXIMO^{7} software.

Hendrix, Michael.- Atlantic States Marine Fisheries Commission: member of the Atlantic sturgeon Technical Committee.

Millard, Michael.- Served on the U.S. Atlantic Salmon Assessment Committee, the technical body which responds to Atlantic salmon assessment tasks defined by the U.S. section to North Atlantic Salmon Conservation Organization (NASCO).

Millard, Michael. - Atlantic States Marine Fisheries Commission: member of horseshoe crab Technical Committee, and serves as chair of horseshoe crab Stock Assessment committee.

Selmer-Larsen, Kim.- Served on the Great Lakes Disease Committee to represent Region 5 relative to disease issues affecting the Great Lakes.

Other Significant Committee Participation:
Barbash, Patricia.- Served on the Maine Fish Health Advisory Board to make recommendations to the Maine commissioners relative to fish health issues impacting wild Atlantic salmon populations and commercial aquaculture.

Barbash, Patricia.- Served on the New England Salmonid Health Committee to make recommendations to the New England Atlantic Salmon Commission (NEASC) relative to fish health issues impacting the New England states.

Hendrix, Michael.- Served on the Exotic Disease Containment Committee: Chaired a committee to develop contingency plans for potential detection of Infectious Salmon Anemia virus (ISAv) at Craig Brook and Green Lake National Fish Hatcheries.

Hendrix, Michael.- Served on the Aquaculture Containment Working Group to develop a HACCP program for the Maine aquaculture industry to prevent escapement of industry-reared fish from freshwater hatcheries and marine sea-pens.

Study Number: LM-00-06

Study Title: Comparative efficacy of three potential anesthetics applicable to Atlantic sturgeon *Acipenser oxyrhinchus* and their physiological consequences

Principal Investigators: Wade Jodun - Northeast Fishery Center; Steve McCormick and Amy Mockel - Conte Anadromous Fish Research Center
Co-investigators: Dr. Mike Millard, Jerre Mohler, - NEFC; Tonia Barton - Penn State University.

Background and Justification:

The development of captive propagation and culture technology for Atlantic sturgeon *Acipenser oxyrinchus* is currently underway at the U.S. Fish and Wildlife Service=s Northeast Fishery Center (NEFC), Lamar, PA with the goal of producing and disseminating a hatchery manual for the species. Many experimental and field investigations require handling fish for a wide array of procedures from tagging to assessing the state of gonadal development and determinating spawning readiness via biopsy. Anesthetics have routinely been employed to subdue fish during these procedures and to alleviate stress and reduce handling associated injury. Metomidate, the methyl derivative of propoxate, has been successfully employed to anesthetize Atlantic sturgeon for experimental purposes but is not yet FDA-approved for use on potential food fish. The compound has been shown to have few adverse side effects and has been used for certain fisheries applications in Canada. In general, information pertaining to anesthetizing Atlantic sturgeon is lacking in published literature. Therefore the need exists to determine safe and effective concentrations of anaesthetics that produce rapid induction, quick recovery and which are non-toxic.

Study Objectives:

(1) Assess the suitability of 3 anesthetics for Atlantic sturgeon: clove oil, MS-222 and metomidate. (2) Determine the concentrations of clove oil, MS-222 and metomidate that will minimize time to induction and time to recovery. (3) Assess the suitability of these anaesthetics for prolonged exposure. (4) Determine if exposure to specific anaesthetic conditions mitigates or induces physiological stress.

Materials and Methods:

In order to facilitate tracking individual fish, all fish were tagged using numbered floy tags prior to the initiation of the study. In **Phase 1** (Range Testing) approximately 200 sturgeon (FY 98 yr-class) were pooled and groups of 12 fish, selected at random, were individually exposed to clove oil (50 - 500 ppm); MS-222 (25 - 300 ppm); and metomidate (5 -25 ppm) at 5, 10 and 15 ^{o}C. Times to induction, recovery and 48 hour mortality were recorded. Fish were given a 1-week recovery period and the experiment was replicated. In **Phase 2** (Prolonged Exposure) juvenile sturgeon were randomly assigned one of the three previously tested anesthetics. Groups of 12 randomly selected fish were then individually exposed to the concentration determined from Phase 1 to be the most effective for their respective anesthetic for periods of 10, 15 and 20 minutes at 5, 10 and 15 ^{o}C. Fish were then returned to fresh water where recovery times and 48 hour mortality were recorded. Fish were given a 1-week recovery period and the experiment was replicated. For **Phase 3** (Stress Assessment) juvenile sturgeon were randomly selected and lots of 10 fish were individually exposed to one of the four following treatments at the most effective concentration determined from Phase 1: (1) Clove oil. (2) MS-222 un-buffered. (3) Metomidate. (4) Water as the control. Stress assessments were conducted at both 5 and 15 ^{o}C. Following treatment, 1 mL of blood was drawn from the caudal vein. Hematocrit was determined immediately by centrifugation. Plasma was drawn off and stored at -20^{o}C for future analysis of cortisol and glucose. Plasma cortisol was measured using radioimmunoassay and plasma glucose was determined colorimetrically using Sigma Diagnostics ortho-toluidine reagent.

Results:

Phase 1: optimal dosages for Metomidate and MS-222 at 5, 10 and 15 ^{o}C were 15 and 200 ppm, respectively. Optimal dosage for Clove oil was 200 ppm at 5 ^{o}C and 100 ppm at 10 and 15 ^{o}C. Metomidate fish required the lowest dosage but took the longest to recover. An inverse relationship between water temperature and recovery times was observed such that colder temperatures resulted in longer recovery times. **Phase 2:** No mortalities resulted from exposing fish to the most effective concentration determined from Phase 1 for up to 20 minutes. However, as exposure time was increased recovery time increased exponentially. **Phase 3:** Stress assessment has not yet been completed

Study Number: LM-01-01

Title: Determination of gonadal maturity in 5 yr-classes of domestic Atlantic sturgeon

Principal Investigator: Jerre W. Mohler-NEFC
Co-investigator: Joel Van Eenennaam -UC Davis

Background and Justification:

From 1993 to 1998, NEFC successfully spawned Atlantic sturgeon (ASN) from the Hudson River resulting in production of 5 year-classes of domestic stock. Currently in existence at NEFC, there are about 700 individuals comprising the five yr-classes. As part of the long-term commitment to refine culture techniques for this species, NEFC is developing domestic broodstock populations at the Lamar, PA facility. This broodstock program is important for two reasons: (1) application of culture technology to potential commercial aquaculture ventures and (2) maintenance of a pool of broodstock available for use in restoration stocking programs should the necessity arise. Currently, it is not possible to accurately determine gender in sub-adult ASN by external characteristics, therefore internal examination is necessary. It is important to identify gender in potential broodstock because there is no information available concerning the influence of environmental conditions on sexual maturation/ovulation in domestic ASN broodstock. Therefore, once gender has been determined in domestic stocks, replicated experimentation is possible to determine the best course of action for controlling female maturation and spawning.

Study Objectives

In November, 2000, we will visually and histologically examine gonadal development via biopsy in at least twelve individuals from each of NEFC=s 5 yr-classes of ASN and permanently identify individuals using PIT tags. Additionally we will perform a variety of body characteristic measurements on sampled fish.

Materials and Methods

Twelve individuals from each of NEFC=s 5 yr-classes of ASN will undergo biopsy at Lamar for gonad sampling in November, 2000. Each fish will be anesthetized using metomidate at 15 ppm then placed ventral side up on a stretcher and sustained with a water-tube during the biopsy. An incision of the necessary length will be made to view the gonad and to extract gonadal tissue samples. Tissue samples will be preserved in 10% formalin upon extraction. Landmarks for visual sex determination include: (1) presence of a distinct groove (ovarian groove) on the lateral side of the ovary for females and (2) a narrow layer of solid tissue (as opposed to adipose) on the dorsal part of the male gonad. A section of gonadal tissue will be removed and sent to UC-Davis for histology and analysis of gonadal development. All fish will be photographed, weighed, measured and PIT-tagged for future reference. All biopsied fish will receive Liquimycin antibiotic at a dosage of 20 mg/kg active terramycin. Aerial photos of the ventral surface of examined fish will be digitized to facilitate accurate measurements of a variety of features and landmark distances. Measurements will be subject to Discriminant Function Analysis (DA) to investigate possible differences between sexes.

Results

Only 2 of NEFC=s 5 year-classes of ASN were examined. Of the 12 six-yr-old ASN evaluated and PIT tagged, three were determined to be females with oocytes in an early (pre-vitellogenic) developmental stage. Out of 12 five-yr-old ASN evaluated, none were able to be conclusively sexed even by histological evaluation. This study will be on-going for a number of years since it was found that most year classes maintained at NEFC are too immature for gender determination.

Study Number: LM- 01-02

Title: Analysis of Lipofuscin pigment concentrations in brain tissue from progressive size classes of horseshoe crabs (*Limulus polyphemus*), as a possible indicator of age.

Principal Investigator: Kim King, Northeast Fishery Center (NEFC)
Co-Invest/Cooperators: Stew Michels, Delaware Natural Resources and Environ. Cons. (DNREC), J R. and C. McConaugha Old Dominion Univ.- VA; Vicki Blazer, USGS/BRD Leetown-WV

Background and Justification:

Recently, much attention has centered on the management of the horseshoe crab *Limulus polyphemus* fishery, due to overexploitation, the need for a sustainable biomedical fisheries and the importance of horseshoe crab eggs as a food source for migratory shore birds. Available data necessary to quantitatively assess the status and dynamics of the population is uninformative and not well developed. Standardized data collection such as spawner and egg count surveys, fishery catch and effort, estimates of natural mortality rates, growth rates, fecundity, etc., is critical to guide the management decisions and policies which will serve to protect the species. In January of 2000, the Horseshoe Crab Stock Assessment Committee (SAC) described and recommended appropriate population dynamic models and assessment surveys to meet these data needs.

Due to the current inability to age *Limulus*, the catch-survey model, applied to species whose age structure is unknown, was recommended. The SAC notes that the development of a reliable aging technique may permit alternative, perhaps better assessment techniques for the future.

Presently, various field observations are used to approximate the age of juvenile and adult horseshoe crabs. Data collected from observations include, the number of mating scars on the shell and the extent of shell erosion, presence/absence of trabeculae, size of dorsal spines and posterior projections of the prosoma (fused head and thorax) and opisthosoma (segmented posterior portion), and the number of annual growth rings in the shell of epibionts attached to adult *Limulus*. Approximating the age of crabs however is a subjective procedure. The ability to age crabs accurately is essential to completely understand the dynamics of the horseshoe crab population. John R. McConaungha, Associate Professor of Oceanography, Old Dominion University, is currently researching a possible aging technique for the Delaware Bay blue crab. The technique involves using a histological procedure to determine the number and size of lipofuscin granules in the olfactory lobe of the crab brain and has been successful in correlating lipofuscin concentrations with the size of the animal. It has been shown that size and number of lipofuscin granules in the olfactory lobe of the American lobster and carapace lengths were significantly related to age. Large lipofuscin concentrations have been found in several areas of the horseshoe crab brain, particularly in the anterior ganglions. These results suggest the possibility that the lipofuscin technique, if standardized, can differentiate cohorts in natural populations of the horseshoe crab.

Study Objectives:

Quantify the variability present in lipofuscin concentrations in brain tissue from progressive size classes of horseshoe crabs.

Materials and Methods:

Horseshoe crab specimens will be collected from Delaware by Stew Michels, Delaware Natural Resources and Environmental Conservation (DNREC), and preserved in 10% formalin. Brain tissue samples will be obtained from 200 crabs, approximately 20 females per size class. Size will be classified as carapace width, to include: <100mm; 100-125mm; 125-150mm; 150-175mm; 175-200mm; 200-225mm; 225-250mm; 250-275mm; 275-300mm; >300mm. Two laboratories, Old Dominion University, Norfolk Virginia and the Northeast Fishery Center, Lamar, PA will divide up the raw materials and simultaneously conduct standardized processing to quantify lipofuscin concentration. The dissection process and histological procedure will be conducted according to accepted, published procedures. Image analysis and quantification of lipofuscin will be conducted at the USGS/BRD Leetown laboratory.

Results

The study is on-going.

Study Number: LM-01-04

Title: Growth comparison of Atlantic salmon parr to determine the effectiveness of three commercial diets

Principal Investigator: Wade Jodun; Northeast Fishery Center
Co-Investigator: Coja Yamashita and Kenneth Thompson; Lock Haven University

Background and Justification:

Since the late 19th Century, federal and state agencies have been involved in rearing Atlantic salmon *Salmo salar* for restoration efforts in the northeastern U.S. with the goal of re-establishing self-sustaining populations to native waters. One component of the ongoing recovery effort includes captive propagation of fish for stocking. Diet is perhaps the single most important factor affecting fish growth and maintaining fish health. Although there is a considerable history of Atlantic salmon culture, little has been written on the nutritional requirements of the species. It was not until the formulation of U.S. Fish and Wildlife Service=s (USFWS) ASD2-30 that any competitively priced Atlantic salmon diet resulted in consistent, satisfactory feed conversion, good growth and acceptable survival at Service facilities (Michael Hendrix, USFWS, personal communication). However, an increase in incidents of poor survival, reduced growth and signs of nutritional deficiencies as well as quality control issues resulted in the USFWS abandoning the diet in 1996. Presently, the USFWS employs a variety of commercial diets with no empirical evidence to suggest which diet promotes maximum growth, survival and feed conversion.

Study Objectives:

We compared three readily available commercial diets for differences upon growth, survival, and feed conversion in Atlantic salmon parr over a 182-day period during 2001.

Materials and Methods:

A pooled lot of 315 Atlantic salmon (mean weight = 1.6 g) were randomly distributed among nine 60-liter circular tanks to a density of 35 fish per tank. Following distribution, tank biomass was adjusted with a precision of 5%. Fish were then acclimated to experimental conditions for 72 hours before feeding began. At the onset of feeding, each tank was randomly assigned one of three diets treatments having three replicates each. The feeds evaluated included: Hi Pro Fry (Corey Feed Mills LTD, Franklin, New Brunswick); Nutra Plus Salmon (Moore Clark, Vancover, British Columbia); and Salmon Starter (Zeigler Brothers, Gardners, PA). Feed was delivered at 0800, 1200 and 1600 hours at a rate of 2.0% body weight per day. Biomass was measured and rations were adjusted at 2-week intervals following the initial inventory to compensate for weight gains. Following data collection, each tank received a prophylactic 1.0% salt treatment to avoid potential disease problems which could result from handling stress. Rearing continued for 182 days. At the conclusion of the 26-week experiment, fish were not fed for 48 hours, at which time 20 fish from each tank were randomly captured and frozen. Frozen whole-body samples from each tank were pooled, homogenized and analyzed for crude protein, lipid, ash and moisture (New Jersey Feed Laboratory, Inc, Trenton, NJ). For statistical purposes each tank was considered a fixed sampling station from which multiple measurements were collected over time. Therefore, the effect of diet on growth was tested with a repeated measures split plot ANOVA. Mean weight per fish was treated as the response variable and diet as the treatment. Tank means were used as experimental units in all analyses to avoid problems associated with pseudoreplication. Overall feed conversion, survival and whole-body composition were analyzed by one-way ANOVA. Survival and whole-body composition data were expressed as percentages and, therefore, arcsine transformed prior to analysis. When significant differences were detected, Tukey-adjusted means comparisons were employed to test for differences among diets.

Results:

After 182 d of rearing, no significant differences in mean weight ($P = 0.35$) or survival ($P = 0.08$) were noted for Atlantic salmon fed either Moore Clark Nutra Plus Salmon, Corey Feed Mills Hi Pro or Zeigler Brothers Salmon Starter diets. Final whole body composition of fish did not vary significantly in terms of levels of crude protein ($P=0.707$), moisture ($P=0.152$), lipids ($P=0.223$) or ash ($P=0.296$) among diets. However, overall feed conversion was affected ($P=0.014$) by diet with Moore Clark producing the most efficient conversion.

Study Number: LM-01-05

Title: Comparison of mortality between calcein-marked and unmarked Atlantic salmon fry stocked in the Sheepscot River, Maine

Principal Investigator: Jerre W. Mohler-NE Fishery Center (NEFC)
Co-investigators: Mike Millard-NEFC; David L. Perkins-R5; Tom King-CBNFH

Background and Justification:

Millions of Atlantic salmon (ATS) fry are stocked throughout river basins in Maine each year, requiring. tremendous effort on the parts of state and federal agencies, and volunteers. A major obstacle to evaluating the performance of these fry has been the lack of a practical technologies for marking fry. Recent advances in the use of calcein to produce an externally-visible mark now offers a solution. The (NEFC) began experimentation with calcein in 1995 by immersing non-feeding fry in calcein solutions. Early tests showed that fin rays of ATS became labeled with calcein, but treated fish exhibited high variability in mark quality. Subsequent experiments led to development of a much more efficient method (osmotic induction) of applying the calcein mark to large numbers of fry. Using this method, marking can be performed on batches of fish in only 7 min. In the hatchery. Calcein detection devices developed at NEFC have been used to reliably detect calcein marks in over 90% of 3-yr-old salmon which were immersed as non-feeding fry. Mark detection is instantaneous, non-lethal, and requires a minimum of equipment. The equipment is portable and compatible with field conditions and requires only that an area of subdued light is present when actual detection takes place. An important question that must be answered concerning new marking techniques is whether survival is similar between marked and non-marked fish released into the wild. Results will help assess the utility and practicality of using calcein to mark fry as part of the evaluation program of ATS recovery in Maine.

Study Objectives

A total of 60K calcein-marked and non-marked ATS fry reared at CBNFH will be evaluated for survival in fall, 2001 after being stocked as non-feeding fry in the Sheepscot and Narraguagas Rivers, ME.

Materials and Methods

The study was conducted in the W. Branch of the Sheepscot River and Shorey Brook area of the Narraguagas River. The majority of fry (about 50,000) were stocked into the W. Branch Sheepscot. Fry were stocked according to normal practices except that half of all fry stocked at each location were calcein-marked following procedures developed by NEFC. In early fall, young-of-year (YOY) were electrofished from multiple sites in the W. Branch using backpack shocking units and the number of marked vs. non-marked fry were compared. Captured fry were examined with a battery-operated calcein detection device to determine mark status. All non-marked fish were fin-clipped for genetic analysis to determine if any non-marked fish were wild origin. All fish were released as soon as data were collected. Numbers of marked and non-marked YOY captured from each location were compared using a Replicated Goodness of Fit test (G-statistic) to test the hypothesis that marked and non-marked fry survived from stocking to capture date at a 1:1 ratio.

Results

Of the 13 stations sampled in the W. Branch Sheepscot, 6 had sufficient data for analysis with 5 of those 6 stations showing marked and non-marked fish captured at the expected 1:1 ratio. The 6^{th} station did not fit the 1:1 ratio due to a greater number of non-marked fish accidentally stocked at that station. Replicated goodness-of-fit tests (G-statistic) applied to overall capture data also showed that non-marked and marked YOY were recovered at the expected 1:1 ratio (pending genetic analysis). Some calcein marks were weak and some fish could have been mis-classified. Field detection equipment performed well and resulted in instantaneous mark classification most of the time. The calcein technique has potential as a relatively inexpensive and practical way to perform hatchery product evaluations where a batch mark is adequate. Refinement of the marking technique is needed to produce consistently visible calcein marks in non-feeding ATS fry.

Of the 2 sites sampled in the Narraguagas, one yielded recovery of 24 calcein-marked YOY and 28 non-marked YOY, fitting the expected 1:1 ratio. The second site yielded 6 marked YOY and 21 non-marked YOY, and did not fit the expected 1:1 recovery ratio.

Study Number LM-01-06

Title: Effect of Hormone Selection and Water Salinity upon Tank Spawning Performance of American Shad

Principal Investigator: John Fletcher-NEFC
Co-investigators: Mike Millard-NEFC; Steve McCormick-Conte Anad. Fish Lab (USGS-BRD)

Background and Justification:

The U.S. Fish and Wildlife Service and partners in the Susquehanna R. Anadromous Fish Restoration Committee (SRAFRC) have been involved in the restoration of American shad (AMS) to the Susquehanna R. for a number of years. In 1998, the Northeast Fishery Center (NEFC) began a cooperative effort to develop and conduct tank spawning technology to establish self-sustaining populations of AMS imprinted to the W. Branch of the Susquehanna R. and to augment egg production for PA Fish and Boat Commission (PFBC). Techniques using hormone implants to induce natural tank spawning of AMS have been under development since 1993 by MD Dept. of Nat. Resources (Manning Hatchery) and for the past several years at Waldoboro Shad Hatchery in Maine. Beginning in 1999, AMS restoration efforts for the Roanoke R. by Edenton Natl Fish Hatchery and N. Carolina Wildlife Resources Comm. (Watha Hatchery), have employed tank spawning techniques. Results from different tank spawning efforts not been consistent. Development of reliable tank spawning protocols will save money, manpower, and time as compared to traditional strip spawning.

Objectives:

Hormone evaluations.- We will compare effect of 3 hormones upon AMS tank spawning as measured by number of eggs produced/day/female. *Water salinity evaluations*.- We will determine survival in low salinity vs fresh water, the impact of water salinity upon reproduction, and the impact of water salinity upon egg size. *Stress evaluations*.- We will examine blood plasma of AMS taken at various intervals to determine levels of stress. *Tank spawning of American shad at Conowingo Dam*.- We will determine if AMS egg production is improved by eliminating transport to NEFC and utilizing a spawning system adjacent to the Conowingo Dam. Blood plasma of AMS will be collected pre- and post- spawning to measure stress indicators.

Methods:

Hormone study.- We conducted trials to assess effectiveness of 3 hormone implants for spawning of AMS: (1) Ovaplant 7 L 150 (Syndel Lab. Ltd.) (2) Ovaplant 7 S 150 (Syndel Lab. Ltd.) (3) Reproboost 7 (VeriPharm LLC). Adult AMS were collected at the Conowingo Dam and transported to NEFC on 5 occasions from May 3-29. After hormone implantation at NEFC, eggs were collected each morning and enumerated prior to incubation. *Salinity and stress study*.- Brood stock collection and transport was similar to that above. For trials, AMS were implanted prior to transport with VeriPharm pellets. Upon arrival, blood was collected from 8 shad of each sex for analysis of stress levels. One treatment used water at 3 ppt salinity while the second was was supplied with fresh water. Data collection and egg processing was conducted as described above. For the 2nd and 3rd shipments blood plasma was collected post-spawn (96 h) from 8 males and females from each treatment. Spawning data was evaluated by ANOVA (alpha of 0.1) and blood chemistry was compared with Dunnett=s t test (alpha of 0.05). *Tank spawning at Conowingo Dam*.- VeriPharm implants were used in10 spawning trials with 36 males and 24 females (3:2) per event. On 3 occasions (early, mid and late season) blood was collected prior to hormone implantation and at post spawning from 10 female AMS in each trial.

Results:

Hormone study.- No differences were found in egg production between hormone treatments. A difference in peak spawning day was found between Ovaplant L and Veripharm LLC with the Veripharm implant producing a lower peak spawning pulse on days 3 and 4. Overall, better quality eggs were produced on days 3 and 4. *Salinity and stress study*.- AMS survival, egg production, and egg viability was greater in the 3 ppt salinity treatment as compared to fresh water. Blood chemistry analysis of AMS held in fresh water showed they were under greater post-spawn stress than those held at 3 ppt salinity. *Tank spawning at Conowingo Dam*.- Egg production was similar but egg viability at 34 % was greater than that at NEFC (22%). Blood chemistry analysis showed that post-spawn AMS were under some stress but not to extent of those spawned in freshwater at NEFC. *Observations*:

About 8.5 million eggs were collected from 965 AMS of which 1.6 million were viable. NEFC reared and stocked 307,000 fry into Bald Eagle Ck, W. Branch - Susquehanna R. with marks at days 3, 6, 9, and 15. The PA Fish and Boat Commission was provided 5 million shad eggs.

Study Number LM-01-07

Title: Mortality Associated with Catch and Release Angling of Striped Bass in the Hudson River

Principal Investigator: Michael J. Millard- NEFC
Co-investigators: Jerre W. Mohler-NEFC; Andrew Kahnle, Kathryn Hatalla-NY Dept. Of Environmental Conservation; Amanda Cosman-New Eng. Interstate Water Pollution Control Comm.

Background and Justification:

Catch and release fishing commonly occurs in recreational fisheries, including the striped bass *Morone saxatilis* (STB) fishery of the Atlantic Coast. The contribution of catch and release to overall fishing mortality is often not estimated. Recent national recreational fishing survey reports indicate that STB anglers released over 90% of their catch in 1997/98. Consequently, hooking mortality may contribute substantially to fishing mortality in the Atlantic coast STB fishery. Estimates from the National Marine Fisheries Comm. recreational fishery survey indicated that over 16 million, 15 million, and 12.5 million STB were released in 1997-1999, respectively. The ASMFC currently assumes a 9% hooking mortality rate for STB. This rate infers a mortality of over 1.1 million released fish along the Atlantic coast each year. These estimates of hooking mortality exceed the estimates of commercial harvests for the same years.

Restoration of the STB fishery on the east coast has increased opportunities for recreational and commercial fishing. Fishery managers must routinely monitor sources of mortality and implement management actions in order to maintain this fishery. Therefore, an evaluation of hooking mortality for STB in the Hudson River is necessary. This study will provide information necessary to determine the contribution of hook and release mortality to the overall mortality rate in the Hudson River STB fishery. Results will be useful in developing guidelines for reducing mortalities of released fish and formulating regulations designed to reduce the non-consumptive mortality rates associated with recreational fishing. This information is particularly important given that the fishery primarily targets one of the largest concentrated spawning populations of STB in the Hudson River.

Methods:

Striped bass were collected from the Hudson River upstream from the Kingston-Rhinecliff bridge, north of Kingston, NY, in a popular angling area known as the Kingston Flats. Volunteer anglers were recruited to provide angled fish between April 30 - May 16, 2001. Participating anglers reported to an anchored project boat upon arrival at the site each day and received bait (primarily alewife*)* and a supply of hooks. Each angler boat was randomly supplied with either traditional straight-shanked AJ@ hooks or non-offset circle hooks and requested to use the assigned hook-type throughout the day.
Anglers used live or chunk bait and fished the bait in any manner they chose within the 2km reach of project boundaries. Three or four transport boats with aerated, flow-through live wells remained in contact with anglers. Immediately after hooking a fish, a transport boat was summoned to take the fish to a shore-based holding tank system. Data recorded for each angled fish included playing time, transport time, hook type, bait type, line weight/test, hook location, and presence of bleeding. Angled fish received a numbered T-bar tag before transport to the holding tank system. Nine holding tanks were provided with flow-through river water at a turnover rate of 50% total volume per hour.

Control fish were captured from the same area via electro-fishing boat and were tagged and transported similar to angled fish. All fish captured on a given day were placed in the same holding tank with a vacant tank used each day to prevent mixing fish from different days. Fish were held for 5 days, with visible mortalities removed and recorded daily. After the 5-day holding time, all remaining fish in a tank were removed, measured, and recorded as being (1) angled/control and (2) male/female and (3) alive/dead. Survivors were released back into the river. A subset of dead angled fish were necropsied to assess any gross physical trauma in the esophagus and surrounding tissues.

Results:

The overall estimate of hooking mortality for both hook types combined was 14%. The mortality rate for circle hooks was 5%, whereas that for J-hooks was 16%. The independent estimates provided by additive rates and the conditional rate estimator were similar. Hook location and the occurrence of bleeding were the most influential variables in determining the probability of death of a hooked and released fish. Inspection of 14 of the 26 deceased angler-caught fish suggested that hook-related damage to the esophagus and surrounding organs, including the heart, led to hemorrhaging and was probably the primary cause of death.

Study Number: LM-01-08

Title: Preliminary evaluation of calcein absorption via the gastrointestinal tract of juvenile Atlantic salmon

Principal Investigator: M. Lisa Moss-Northeast Fishery Center (NEFC)
Co-investigators: Jerre W. Mohler, John W. Fletcher-Northeast Fishery Center

Background and Justification:

Previous studies conducted at NEFC have demonstrated the potential for use of calcein ($C_{30}H_{26}N_2O_{13}$) to mass-mark fish for hatchery product assessment and long-term stock management. These efforts have focused on the use of this compound as an immersion bath for early life-stage Atlantic salmon (ATS). However, physical immersion of fry has produced variability in uptake, influenced by chemical concentration, exposure time, temperature and short-term retention of the mark. Injection can be used to produce calcein marks but requires handling of individual fish. In addition, both procedures can induce stress due to increased handling and abrupt chemical exposure. In the current study, we investigate an alternative technique for evaluating the efficacy of calcein absorption via the gastrointestinal tract of ATS parr and developing smolts. Results of the current study will be used as a foundation for eventual incorporation of calcein into a formulated fish feed.

Study Objectives:

(1) In June-July 2001, we will develop and administer calcein or placebo agar pellets to 114 yearling ATS, **(2)** prepare two different dosages for calcein mark induction, and **(3)** perform follow-up evaluations of subject fish to assess calcein mark retention as affected by growth.

Materials and Methods:

Two calcein dosages, 0.5 mg/kg and 3.0 mg/kg were used to compare observations of chemical deposit and fluorescence intensity. Single dosages were calculated based on the mean body weight (20.1 g) of 114 yearling ATS. Calcein was introduced into a pre-gelatin mixture of standard Bacto-Agar as 0.5% calcein stock solution. Individual calcein doses as single pellet dosages were prepared from perforated PVC molds set in petri dishes with circular openings of equal size and distribution. Based on the calcein requirement (mg/fish) and pellet volume, total calcein in agar solution needed to cover the mold was calculated and proportionately added to agar solution. Subject fish were anesthetized using MS-222 and held vertical with mouth held open while a gelatin pellet was guided into the abdominal cavity with a modified dropper and catheter tube. Fish accurately identified to have expelled the pellet upon recovery received a repeat dose. Fish were segregated according to dosage and evenly distributed among nine, 0.6-m-diameter circular tanks fitted with top screens plumbed to provide flow-through ambient water. Mortalities were frozen in water and included in the evaluation. Within 1-2 weeks, fish were evaluated in a dark room for evidence of marks using a calcein detection device (patent pending).

Results:

Following a single calcein dose, 85% of exposed ATS exhibited readily detectable marks of low-high brilliance. Thirty-seven percent and 75% of fish exposed to 0.5 and 3.0 mg/kg calcein respectively, had marks on the mandibles, opercula, scales, fin and tail rays, caudal peduncle and keel area of medium-high brilliance. Marks on scales and rays appeared as intense localized dots. In late January 2002, a sub-sample of 24 smolts exposed to 3.0 mg/kg was evaluated and 37.5% retained brilliant scale and fin ray marks while 12.5% retained brilliant fin rays, but absent or dim scale marks. Follow-up evaluation of these smolts will be done to assess long-term durability of a single dose as affected by growth. Further studies should investigate repeat dosages within a range of calcein concentrations and monitor a uniform ATS population over a long-term period (1 yr +).

OTHER BIOLOGICAL AND RELATED INVESTIGATIONS PERFORMED:

LM01A **Fish Health Inspection/Monitoring/Diagnostic Services.** - The Lamar Fish Health Center processed 365 laboratory cases in fiscal year 2001. Region 5 has a very extensive fish health monitoring and inspection program that permits continual surveillance of the health status of the stocks, some of which have been identified as distinct population segments (DPS) under the Endangered Species Act (ESA). The Fish Health Unit had 27 inspection cases, which included 14 that were conducted, as outlined in the Service Fish Health Policy, as virology lab services only for non-Service entities. These statistically based fish health examinations are essential to prevent the spread of fish diseases through fish and/or egg transfers and are necessary to enable facilities to comply with regulations on transporting and releasing fish. In addition to the 167 monitoring cases involving examination of fish, 3 Service facilities provided 37 water monitoring cases, where water from rearing units is examined by the water filtration method, a very effective protocol for diagnosing furunculosis before an epizootic occurs and for evaluating efficacy of water treatment. In fiscal year 2001 twenty-nine laboratory cases were diagnostic examinations, where moribund fish were examined and tested to determine the cause(s) of mortalities and other problems and recommendations for resolution were provided. As a cooperator in various fisheries projects within the region, the Fish Health Center also examined aquatic invertebrates (7 cases) which involved fisheries management activities with sandworms, bloodworms, and freshwater mussels and their potential to spread fish pathogens.

LM01B **Licensing agreement for calcein detection devices**.- In 2000, NEFC submitted a patent application for both a bench-top and hand-held calcein detection device which will make it feasible to quickly and efficiently detect fluorescent marks on fish under rigorous field conditions without the need for a microscope. In 2001, negotiations with Western Chemical Co. were undertaken by the USFWS for granting exclusive licensing to Western Chemical to produce and market the devices, and an agreement has been signed.

LM01C **Ozone water treatment plant construction**.- Construction was completed on the ozone water treatment plant for the Intensive Culture Unit. The system is computer-controlled to treat filtered water for a minimum of 7 minutes with residual ozone concentration of 0.2 mg/L prior to ozone destruction. The system is self-regulating and self-adjusting to flows between 350 - 2250 Liters/minute. Utilization of the system will provide a research environment which will not be subject to disease-producing levels of pathogens. The system is also designed to allow experimentation on the kinetics and pathogen control effectiveness of ozone water treatment.

LM01D **Participation in the National Wild Fish Health Survey**. - This project, launched in 1997, continues to involve all nine Service fish health centers nationwide incorporating standardized diagnostic techniques and data management methods to ensure comparability. In fiscal year 2001, the Fish Health Unit initiated 98 cases for the Survey, in which 3,961 fish (44 different species) from a total of 72 sites were examined and efforts continued to enter completed cases into the NWFHS database. This database which is capable of single and double queries based on either fish species or fish pathogens, is now accessible via the Internet on the service website. The National Wild Fish Health Survey is partnership driven and fiscal year 2001 enabled the Lamar Fish Health Center to conduct cooperative work with Stratus Consulting and the New York Ecological Services Field Office on contaminants in the Hudson River, Penn State University on whirling disease in feral trout populations in Pennsylvania, Shenadoah National Park on wild, unmanipulated population inventories, and several state natural resource agencies on developing broodstocks from feral populations. Outreach activities to increase awareness of the Survey and involve other Fish and Wildlife Service programs included demonstrations of the database on the Internet to the Region 5 Regional Directorate Team as well as development of a NWFHS Outreach Committee coordinated out of the Washington Office.

LM01E **Incidence and Prevalence of Infectious Salmon Anemia in Sea Run Penobscot River Atlantic Salmon held at Craig Brook NFH for Broodstock**. - Due to the recent finding of Infectious Salmon Anemia virus (ISAv) from Atlantic salmon in maritime culture in waters of Maine (a first isolation in United States), a surveillance protocol for screening sea-run Penobscot River Atlantic salmon as they are captured and brought to Craig Brook National Fish Hatchery was initiated to determine incidence in this population. For logistics, a sub-sample (n=68) of fish were sampled non-lethally (blood) via specific DNA segment amplification and identification (polymerase chain Reaction, PCR) and after the protocol was established another assay, culture on SHK-1 cells, was also performed (n=50). One fish tested positive by PCR and was segregated from the population. As a tool for managing this virus at the facility, the entire population (n=494) was similarly screened, following cohabitation and prior to spawning. All tested negative and no isolation / quarantine of eggs was deemed necessary.

LM01F **Ongoing Participation in Maine Fish Health Advisory Board concerning ISAv Issues**.- The Maine Fish Health Advisory Board serves as a scientific advisory board to the state Commissioners. The group, containing a representative from the Lamar Fish Health Center, is very heavily involved with Infectious Salmon Anemia virus (ISAv), as well as other fish health issues related to private aquaculture and wild resources. The Center has established a monitoring program for all sea-run Atlantic salmon mortalities as has the Maine salmon industry initiated an intensive ISAv monitoring program. The Committee has reviewed and participated in a US Dept. of Agriculture (USDA), Animal Plant Health Inspection Service (APHIS) Indemnification Program for eradication of ISAv in Maine. Part of the program involves depopulation and fallowing of all salmon net pens in Cobscook Bay, where ISA has been a problem for the private aquaculture industry for over a year. Due to the low numbers of the distinct population segments (DPS) of Atlantic salmon, it is vital that the impacts of this and other diseases are held to a minimum.

LM01G **U.S. Fish and Wildlife Service Fish Health Procedures Handbook** -In cooperation with all eight other USFWS fish health centers, and in collaboration with the American Fisheries Society - Fish Health Section, a procedural manual for Fish Health Inspection Protocols has neared completion. A representative of the Lamar FHC chaired subcommittees to gather and edit procedures for the Sampling, Bacteriology and Quality Assurance Chapters of this Manual. The intent of the AManual@ is to establish a nationally consistent set of protocols for use by all fish health inspectors and diagnostic laboratories when performing fish health inspections of fish culture and aquaculture facilities. The document will be provided to the Fish Health Task Force of the Congressional Joint Subcommittee on Aquaculture (JSA) for their review and adoption as a national standard for Fish Health Inspections. The document will also become and addendum to the AFS/FHS ABluebook@.

LM01H **Quality Assurance/Quality Control for ISAv Samples and Diagnostic Techniques.** - The Lamar Fish Health Center participated in an ISAV assay and procedure QA/QC program with National Marine Fisheries Service University of Maine at Orono, and MicroTechnologies, Inc. during fiscal year 2001. Receiving blind samples collected by UMO from clinical and subclinical experimentally infected fish, as well as negative fish, this exercise is examining the differences between sampling blood (non-lethal) and tissues (kidney/spleen) as well as the accuracies and sensitivities between the PCR and cell culture assays, all conducted with duplicate samples in two laboratories. A total of 60 fish, producing 360 assays are in progress at the Lamar Fish Health center and MicroTechnologies Inc.

LM01I **Fish Health Extension Services** - The Lamar Fish Health Center continues to provide extension services to all federal, state, tribal and private inquiries in the area of fish health. Services provided include verbal consultations, provision of supplies for fish necropsies, antibiotic injections, and vaccinations, and provision of procedural protocols